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PCT.

JC11 Rec'd PCT/PTO 13 MAR 2001

FORM PTO-1390 (REV 10-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: UMDNJ-31060

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/787072

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/20942	13 September 1999	

TITLE OF INVENTION: RIBOSOMAL FRAMESHIFT TARGETS

APPLICANT(S) FOR DO/EO/US: University of Medicine and Dentistry of New Jersey

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1 [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371

2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371

3 [ ] This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f))

4 [ ] The US has been elected by the expiration of 19 months from the priority date (PCT Article 31)

5 [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))

- a. [X] is attached hereto (required only if not communicated by the International Bureau).
- b. [ ] has been communicated by the International Bureau.
- c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)

6 [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).

7 [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))

- a. [ ] are attached hereto (required only if not communicated by the International Bureau).
- b. [ ] have been communicated by the International Bureau.
- c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
- d. [X] have not been made and will not be made.

8 [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9 [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (Unexecuted)

10 [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5))

Items 11 to 16 below concern document(s) or information included:

11 [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98

12 [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13 [X] A FIRST preliminary amendment

[ ] A SECOND or SUBSEQUENT preliminary amendment

14 [ ] A substitute specification.

15 [ ] A change of power of attorney and/or address letter

16 [X] Other items or information

Express Mail Label No. EJ239782343US
PTO 1449

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/787072	PCT/US99/20942	UMDNJ-31060

17. ☒ The following fees are submitted:

JC08 Rec'd PCT/PTO 13 MAR 2001

	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ... $1000.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) ... $100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 1000.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e))	$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	18 - 20 =	0	X $18.00	$
Independent claims	6 - 3 =	3	X $80.00	$ 240.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$
TOTAL OF ABOVE CALCULATIONS =				$ 1240.00
☒ Applicant claims small entity status See 37 CFR 1.27 The fees indicated above are reduced by 1/2.				$ 620.00
SUBTOTAL =				$ 620.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)) +				$
TOTAL NATIONAL FEE =				$
Fee for recording the enclosed assignment (37 CFR 1.21(h)) The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) $40.00 per property +				$
TOTAL FEES ENCLOSED =				$ 620.00
			Amount to be refunded:	$
			charged:	$

a. ☒ A check in the amount of $620.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No 03-3839 A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

Customer No. 26345

SIGNATURE

Kristine L. Butler

NAME

42,376

REGISTRATION NUMBER

Express Mail Label No. EJ239782343US

UMDNJ-31060

JC08 Rec'd PCT/PTO 13 MAR 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:		**Examiner:**	**Not assigned**
Dinman, et al			
		Group Art Unit:	**Not assigned**
International App. No.:	**PCT/US99/20942**		
International Filing Date:	**September 13, 1999**		
US Filing Date:	**March 13, 2001**		
For:	**RIBOSOMAL FRAMESHIFT TARGETS**		

Box Non-Fee
Assistant Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Sir:

This application is the National Stage Application of PCT/US99/20942, which claims priority from US Provisional Application 60/100285, filed by September 14, 1998. This Response is submitted along with the above-captioned application on March 13, 2001.

ABSTRACT

Please disregard the abstract that appears on the coversheet of the PCT application and substitute the following abstract:

-- The invention relates to sequences involved in ribosomal frameshifting in mammalian genes. Specifically, methods of identifying ribosomal frameshift sequences in mammalian genes, and methods of regulating gene expression by modulating ribosomal frameshifting are

disclosed. In addition, a system for identifying a nucleic acid sequence involved in ribosomal frameshifting is disclosed.--

REMARKS

As stated above, Applicants request that this abstract replace the abstract as displayed on the coversheet. Applicants have attached a separate sheet with the above noted abstract.

Applicants believe that this application is in condition for allowance. However, if the Examiner is of the opinion that such action cannot be taken, the Examiner is requested to call the Applicant's attorney at (973) 596-4683 in order that any outstanding issues may be resolved without the necessity of issuing a further action.

In addition, Applicants request that all correspondence be addressed to Intellectual Property Docket Administrator, Gibbons, Del Deo, Dolan, Griffinger & Vecchione, One Riverfront Plaza, Newark, NJ 07102-5497. Also, any fax communications should be sent directly to 973-639-6254.

Any fees due and owing can be charged to Gibbons, Del Deo, Dolan, Griffinger & Vecchione Deposit Account No. 03-3839.

Respectfully submitted,

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RIBOSOMAL FRAMESHIFT TARGETS

Background of the Invention

Maintenance of correct reading frame during translation of mRNA is fundamental to the integrity of the translation process and, ultimately, to cell growth and viability. However, a number of cases have been identified in which translating ribosomes are directed to shift reading frames, a phenomenon referred to as "programmed ribosomal frameshifting". Most of these ribosomal frameshift events have been observed in RNA viruses. Families of mammalian viruses in which ribosomal frameshifting has been observed include retroviruses, coronaviruses, toroviruses, arteriviruses, astroviruses, and paramyxovirus. Plant viruses in which frameshifting has been observed include tetraviruses, and tombusviruses. In fungi, ribosomal frameshifting has been observed in the totiviruses and many retrotransposable elements. Among bacteriophages, ribosomal frameshifting has been documented in T7 and λ. Viral frameshifting events typically produce fusion proteins in which the N- and C-terminal domains are encoded by two distinct, overlapping open reading frames. Ribosomal frameshifting in viruses determines the stoichiometric ratio of structural (Gag) to enzymatic (Gag-pol) proteins, and plays a critical role in viral particle assembly. The study of these ribosomal frameshifts has been important both because of their critical role in viral morphogenesis, and because of the information they provide about the mechanisms by which reading frame is normally maintained.

The cis-acting sequences that promote efficient ribosomal frameshifting in the -1 (5') direction have been well characterized in several viral systems and it has been convincingly demonstrated that the basic molecular mechanisms governing programmed -1 ribosomal frameshifting are almost identical from yeast to humans. Two basic sequence elements are required to promote efficient levels of programmed -1 ribosomal frameshifting. The first sequence element is heptamer sequence, X XXY YYZ (wherein the 0-frame is indicated by spaces) called the "slippery site". The simultaneous slippage of ribosome-bound A- and P-site tRNAs by one base in the 5' direction still leaves their non-wobble bases correctly paired with the mRNA in the new reading frame. The second promoting element is usually a sequence that forms a defined RNA secondary structure, such as an RNA pseudoknot, located within 8 nucleotides 3' of the slippery site, and is thought to increase the probability that the ribosome will shift reading frame in the -1 direction. The number of ribosomes that shift frame is affected by a number of parameters, including the ability of the ribosome bound tRNAs to unpair from the 0-frame, the ability of these tRNAs to rebind to the -1 frame, the relative position of the RNA pseudoknot from the slippery site and the thermodynamic stability of the pseudoknot.

There are a few documented examples in which programmed ribosomal frameshifting is utilized by mRNAs of cellular origin. In *E. coli*, autoregulation of a programmed +1 ribosomal frameshift in the

prfB gene is required for the synthesis of Release Factor 2 (RF2) (Craigen and Caskey, 1986; Craigen et al., 1985; Donly et al., 1990a; Donly et al., 1990b), and a -1 ribosomal frameshift in the *dnaX* gene generates the DNA polymerase gamma subunit (Flower and McHenry, 1991; Blinkowa and Walker, 1990; Tsuchihashi and Kornberg, 1990). In eukaryotic mRNAs, programmed +1 ribosomal frameshifting has been demonstrated in genes encoding ornithine decarboxylase (ODC) Antizyme isolated from rat, mouse, *xenopus*, *drosophila* (Hayashi and Murakami, 1995; Ivanov et al., 1998; Kankare et al., 1997; Ichiba et al., 1995; Matsufuji et al., 1995; Rom and Kahana, 1994), and in the *EST3* gene of *S. cerevisiae* (Lundblad and Morris, 1997). In mammalian cells, the control of ribosomal frameshifting efficiency is autoregulated by ODC Antizyme protein levels (Craigen and Caskey, 1986; Craigen et al., 1985; Donly et al., 1990a; Hayashi and Murakami, 1995; Matsufuji et al., 1995). In yeast cells which lack ODC Antizyme, high concentrations of putrecine and consequently low concentrations of spermidine promote increased efficiencies of frameshifting in the +1 direction (Balasundaram et al., 1994b; Balasundaram et al., 1994a). Thus, the regulation of polyamine biosynthesis demonstrates how programmed ribosomal frameshifting may be used by eukaryotic cellular genes as a post-transcriptional regulatory mechanism. Although there are no known examples of eukaryotic cellular mRNAs which utilize programmed -1 ribosomal frameshifting, certain observations suggest that this mechanism may also be biologically relevant for these cells as well. Certain yeast strains harboring chromosomal mutations which increase the efficiency of -1 ribosomal frameshifting (*mof* = *m*aintenance *of* frame) show cellular defects as well, *e.g.* temperature sensitive cell cycle growth arrest, temperature-sensitive mating defects, mitochondrial defects, sensitivity to translational inhibitors, inability to degrade nonsense mRNAs, and slow growth phenotypes (Cui et al., 1996; Dinman and Wickner, 1992; Dinman and Wickner, 1994). These observations suggest that -1 ribosomal frameshifting may play a role in the regulation of cellular gene expression, and that changes in the efficiency of -1 ribosomal frameshifting may affect cell growth and replication.

Based on the hypothesis that biological systems tend to conserve and use functional molecular regulatory mechanisms, a computer search program was designed to identify consensus -1 ribosomal frameshift signals in large DNA databases. It was found that consensus -1 ribosomal frameshift signals occur with frequencies significantly greater than random in these databases. It was also demonstrated that one of the predicted -1 ribosomal frameshift signals, occurring at the 5' end of the yeast Rasl mRNA, promotes efficient levels of -1 ribosomal frameshifting in the yeast *S. cerevisiae*.

Summary of the Invention

In accordance with the present invention, it has been discovered that gene sequences which have the frameshifting sequences exist in many organisms other than viruses. Frameshifting sequences have been newly identified in numerous yeast, avian, and mammalian sequences.

A computer search was designed to search for consensus -1 ribosomal frameshift signals (motif hits) present in the EMBL virus, *Saccharomyces cerevisiae*, human mRNA, cDNA and Expressed Sequence Tag (EST) databases. These searches found that potential -1 ribosomal frameshifting signals occur at frequencies greater than one order of magnitude above random chance. This result provides strong theoretical evidence for the existence of a subset of cellular genes which are regulated at the translational level by -1 ribosomal frameshifting in eukaryotes, and that this post transcriptional regulatory mechanism is widely used by many different families of viruses as well.

The present invention provides a method of identifying a nucleic acid sequence involved in ribosomal frameshifting. The method comprises 1) searching a database of gene sequences to identify sequences which contain the sequence XXX YYY Z , wherein XXX represents GGG, AAA, TTT or CCC, YYY represents AAA or TTT, Z represents A, T, or C and wherein XXXYYYZ is not AAAAAAA or TTTTTTT; and 2) further searching among those sequences identified in step 1 for a sequence encoding a pseudoknot structure which is within eight nucleotides of the sequence identified in step 1.

The present invention also provides a method of identifying a nucleic acid sequence involved in ribosomal frameshifting, comprising the steps of selecting a gene sequence having a sequence of nucleotides from the group of GGG, AAA, TTT and CCC; selecting said gene sequence having an adjacent sequence of nucleotides from the group of AAA and TTT; selecting said gene sequence having a nucleotide from the group of A, T and C, said nucleotide adjacent to said adjacent sequence of nucleotides; excluding said gene sequence wherein said sequence of nucleotides is AAA, said adjacent sequence of nucleotides is AAA and said nucleotide is A; excluding said gene sequence wherein said sequence of nucleotides is TTT, said adjacent sequence of nucleotides is TTT and said nucleotide is T; searching for an encoded pseudoknot structure which starts within eight nucleotides of said selected gene sequence.

The present invention further provides a system for identifying a nucleic acid sequence involved in ribosomal frameshifting, the system comprising access means for accessing a database of gene sequences; selection means for selecting a particular gene sequence from said database of gene sequences, said particular gene sequence having a sequence of nucleotides from the group of GGG, AAA, TTT and CCC, an adjacent sequence of nucleotides from the group of AAA and TTT, a nucleotide from the group of A, T and C, said nucleotide adjacent to said adjacent sequence of nucleotides, wherein said particular gene sequence is excluded from selection when said sequence of nucleotides is AAA, said adjacent sequence of nucleotides is AAA and said nucleotide is A and said particular gene sequence is excluded from selection when said sequence of nucleotides is TTT, said adjacent sequence of nucleotides is TTT and said nucleotide is T; pseudoknot search means for locating an encoded pseudoknot structure which starts within eight nucleotides of said selected gene sequence.

The present invention also provides a method of regulating expression of a mammalian gene comprising modulating the frequency of ribosomal frameshifting during translation of messenger RNA.

Brief Description of the Drawings

Figure 1: Consensus programmed -1 ribosomal frameshift signal.

Figure 2: Conservation of two frameshift signals in homologous genes from different organisms.

Detailed Description of the Invention

The present invention provides a method of identifying a nucleic acid sequence involved in ribosomal frameshifting. The method comprises searching a database of gene sequences to identify nucleic acid sequences which contain a slippery site and a pseudoknot structure associated with frameshifting. The method comprises first searching for a slippery site, which is identified by the sequence XXX YYY Z, wherein XXX represents GGG, AAA, TTT or CCC; YYY represents AAA or TTT; Z represents A, T, or C; and wherein XXXYYYZ is not AAAAAAA or TTTTTTT. Further searching is conducted among those sequences containing a slippery site for a sequence encoding a pseudoknot structure which is within eight nucleotides of the slippery site sequence.

The slippery site may have any of the following nucleic acid sequences: GGG AAA A, GGG AAA T, GGG AAA C, AAA AAA T, AAA AAA C, TTT AAA A, TTT AAA T, TTT AAA C, CCC AAA A, CCC AAA T, CCC AAA C, GGG TTT A, GGG TTT T, GGG TTT C, AAA TTT A, AAA TTT T, AAA TTT C, TTT TTT A, TTT TTT C, CCC TTT A , CCC TTT T, and CCC TTT C.

The present invention also provides a method of identifying a nucleic acid sequence involved in ribosomal frameshifting, comprising the steps of selecting a gene sequence having a sequence of nucleotides from the group of GGG, AAA, TTT and CCC; selecting said gene sequence having an adjacent sequence of nucleotides from the group of AAA and TTT; selecting said gene sequence having a nucleotide from the group of A, T and C, said nucleotide adjacent to said adjacent sequence of nucleotides; excluding said gene sequence wherein said sequence of nucleotides is AAA, said adjacent sequence of nucleotides is AAA and said nucleotide is A; excluding said gene sequence wherein said sequence of nucleotides is TTT, said adjacent sequence of nucleotides is TTT and said nucleotide is T; searching for an encoded pseudoknot structure which starts within eight nucleotides of said selected gene sequence.

The present invention further provides a system for identifying a nucleic acid sequence involved in ribosomal frameshifting, the system comprising access means for accessing a database of gene sequences; selection means for selecting a particular gene sequence from said database of gene sequences, said particular gene sequence having a sequence of nucleotides from the group of GGG, AAA, TTT and CCC, an adjacent sequence of nucleotides from the group of AAA and TTT, a nucleotide

from the group of A, T and C, said nucleotide adjacent to said adjacent sequence of nucleotides, wherein said particular gene sequence is excluded from selection when said sequence of nucleotides is AAA, said adjacent sequence of nucleotides is AAA and said nucleotide is A and said particular gene sequence is excluded from selection when said sequence of nucleotides is TTT, said adjacent sequence of nucleotides is TTT and said nucleotide is T; pseudoknot search means for locating an encoded pseudoknot structure which starts within eight nucleotides of said selected gene sequence.

Translation of any gene containing frameshift sequences, namely the slippery site and psuedoknot sequences, is potentially regulated by the ribosomal frameshifting mechanism. Consequently, translation of such a gene may be regulated by known methods of altering the frequency of frameshifting, for example, by use of drugs which affect the peptidyl transferase activity. Accordingly, the invention provides a method of regulating expression of a mammalian gene comprising modulating the frequency of ribosomal frameshifting during translation of messenger RNA. In accordance with the method, the frequency of frameshifting may be increased or decreased.

Computer search protocols.

The GenBank *Saccharomyces cerevisiae*, *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Gallus gallus*, *Sus scrofa*, *Drosophila melanogaster*, and Virus divisions, and 2 x 10^4 random sequences of 10^3 bases (G-C content = 50%) were searched using the following algorithmic structure:

Step 1: Search for XXXYYYZ (slippery site) where:

XXX = GGG, AAA, TTT or CCC

YYY = AAA or TTT

Z = A, T, or C

AND XXXYYYZ = AAAAAAA or TTTTTTT.

Step 1 can be implemented by selecting a gene sequence having a sequence of nuceotides from the group of GGG, AAA, TTT and CCC; selecting the gene sequence having an adjacent sequence of nucleotides from the group of AAA and TTT, selecting the gene sequence having a nucleotide from the group of A, T and C, the nnucleotide adjacent to the adjacent of nucleotides; excluding the gene sequence wherein the sequence of nucleotides is AAA, the adjacent sequence of nucleotides is AAA and the nucleotide is A; and excluding the gene sequence wherein the sequence of nucletides is TTT, the adjacent sequence of nucleotides is TTT and the nucleotide is T.

Step 2: Search for a pseudoknot 3' of the XXXYYYZ slippery site motif using the GenoBase program. Further constraints placed on the pseudoknot were:

a. The pseudoknot must begin within 8 nucleotides (NT) of base Z:

b. Stem 1 must have a minimum length of 6 base pairs, containing no more than 1 mismatch, 1 insertion or 1 deletion;

c. Gap 1 (the gap between stem 1 and stem 2) can be no greater than 3 NT in length;

d. Stem 2 must have a minimum of 5 base pairs with only 1 insertion, deletion or mismatch allowed;

e. Gap 2 can be no greater than 3 NT in length;

f. Gap 3 is limited to 100 NT in length.

Step 3: Align motifs found in steps 1 and 2 with an open reading frame (ORF) of at least 50 codons, such that the first base in the slippery site (the first X) is in the third base of a codon. Further, searching in the 5' direction of the motif there must be an in-frame ATG codon before a translational termination signal (TAA, TAG, or TGA). Sequences that satisfied all of these criteria were defined as "motif hits".

Strains, media , genetic methods. and plasmid construction.

E. coli strain DH5 was used for plasmid preparations. and transformations of *E. Coli* and *S. Cerevisiae* were performed (Dinman and Wickner, 1992). YPAD and synthetic complete medium were prepared (Dinman and Wickner. 1994). The *S. cerevisiae* strain JD88 *(MATa ura3-52 lys2-801 ade2-10 trp1)* [L-AHNB] [M_1]) was used for *in vivo* measurements of -1 ribosomal frameshifting efficiencies as described in (Dinman and Wickner. 1992).

pJD160.0 is based on p314-JD86-ter (Cui et al., 1996). with the modification that it contains unique *Bam* HI. *Sma* I and *Kpn* I restriction endonuclease recognition sites 3' of the AUG start codon, and 5' of the *lacZ* gene. This is the 0-frame control plasmid. pJD160.-1 is identical to pJD160.0 except that lacZ is in the -1 frame with respect to the translational start site without any intervening frameshift signal. This is used to measure unprogrammed -1 ribosomal frameshifting. The frameshift signals from the yeast *RAS1* gene was amplified from genomic DNA by polymerase chain reaction (PCR) as described (Costa and Weiner. 1995) using the synthetic oligonucleotide primers shown in Table 1.

Table 1. Oligonucleotid Primers used in this study

Oligonucleotide Primer	Description
5' AAAG*AATTCC*GA**CATGCAGGGAAATCCAAATCAAC** 3' (SEQ ID NO:1)	RAS1 5' Eco RI.
5' CCCC*GGTACC***GTCATCGATGACAACTT** 3' (SEQ ID NO:2)	RAS1 3' Kpn I.

Italicized bases denote added restriction endonuclease recognition sites. Bold bases indicate gene sequence. Underlined bases were added 3' of the slippery site and 5' of the predicted mRNA pseudoknot forming region so that a -1 ribosomal frameshift will direct elongating ribosomes into the original reading frame.

Since the RAS1 frameshift signal is predicted to direct ribosomes into premature termination signals. two additional nucleotides were added in the spacer regions between the slippery sites and pseudoknots of these PCR products such that a -1 frameshift would re-direct ribosomes into the original reading frame. The PCR products were cloned into pJD160.0 to produce pJD160.RAS1. In this construct, a programmed -1 frameshift is required for in order for the *lacZ* gene to be translated.

RESULTS

The program is capable of finding known viral programmed -1 ribosomal frameshift signals.

As a positive control, the program was used to search all 36,556 loci of the GenBank virus division, revealing 1077 motif hits. The program identified almost all of the known viral -1 ribosomal frameshift signals including those that have been classically used to study programmed -1 ribosomal frameshifting. These include Mouse Mammary Tumor Virus, Feline Leukemia Virus, and Infectious Bronchitis Virus. As expected, the program was not able to identify the motif hit in Rous Sarcoma Virus because the Gaps 1 and 2 represented in Figure 1 are larger than allowed by the program. In addition, many motif hits were identified in families of viruses where -1 ribosomal frameshifting has not been described. For example, a frameshift motif appears to be well conserved in the E1B protein large T-antigen mRNA among the adenoviruses, and in the VP16 family of proteins in many of the herpesviruses.

Consensus motif hits occur at frequencies significantly greater than random in the genome databases.

If a subset of cellular genes utilize programmed -1 ribosomal frameshifting, then it may be assumed that the consensus frameshift motifs should be present in the genomes of many different species at frequencies significantly greater than random. To test this, the probability of the random occurrence of a motif hit was determined. The program was run twice against 10^4 randomly generated sequences of 10^3 bases. For technical reasons, the G:C content was set to 50%. This negative control found 41 motif hits in the first run and 42 in the second. Thus, the random frequency of motif hits is 83 per 2×10^7 bases. Searches of the large DNA databases revealed that motif hits occur with frequencies significantly greater than random (Table 2).

Table 2. Summary of search results.

Organism	# Bases Searched	# Motif Hits	Fold > Random
Random sequence	2.0×10^7	83	-
Saccharomyces cerevisiae (yeast)	1.2×10^7	260	5.22
Homo sapiens (human)	9.52×10^7	1055	2.67
Mus musculus (mouse)	2.13×10^7	320	3.62

Rattus norvegicus (rat)	1.14×10^7	103	2.18
Gallus gallus (chicken)	2.37×10^6	57	5.8
Sus scrofa (pig)	1.5×10^6	25	4.02
Drosophila melanogaster (fruitfly)	1.16×10^7	167	3.47
Viruses	3.7×10^7	1077	7.0

The results from the *S. cerevisiae* genome should provide the best estimate of the frequency of motif hits, because 1) it is complete, 2) it is on the same order of magnitude as the random control, 3) it contains the least amount of duplications, and 4) it was sequenced without reading-frame bias. Analysis of this dataset revealed 260 motif hits, approximately 5.2-fold more frequent than random. BLAST analysis revealed that 153 different recognized genes or CDS were represented. Since the yeast genome is estimated to contain approximately 5900 genes, these data suggest that at least 2.55% of the genes in the yeast genome contain at least one consensus programmed -1 ribosomal frameshift signal. Further, since the algorithm limited the size of gap1 and gap2 and disallowed slippery sites of TTTTTTT and AAAAAAA , the data probably represent an underestimate of the fraction of motif hits containing yeast genes.

Frameshift signals appear to be evolutionarily conserved between homologous genes in different species.

If a subset of cellular genes utilize programmed -1 ribosomal frameshifting, then specific frameshift signals would be evolutionarily conserved in homologous genes from different organisms. A preliminary comparison of the locations and structures of motif hits in homologous genes in the different databases reveals cases where nearly identical motif hits appear to be conserved. Two such examples, a comparison of Fibrillin 2 in human and mouse, and of the Sulfonurea Receptor in humans and rat are shown in Fig. 2. It is notable that whereas the slippery sites and stems of the motifs are highly conserved, the lengths of gap3, which are not expected to play a critical role, are variable in both of these examples. Thus it appears that the biologically important elements of the frameshift signals have been conserved, while the unimportant elements have been allowed to drift.

Mutations that have been linked to inherited human diseases correlate with those that are predicted to abolish -1 ribosomal frameshifting.

If programmed -1 ribosomal frameshifting has a biologically relevant function in cellular gene expression, then there should be a correlation between mutations that disrupt frameshifting by altering the -1 ribosomal frameshift signal, and human alleles that have been linked to genetically inherited diseases. This hypothesis predicts that the disease alleles would encode missense mutations, or the addition or deletion of entire codons. A preliminary analysis of the human motif hit database identified four alleles of three genes that fit these criteria (Table 3).

Table 3: Three Human Genes Where Specific Mutations in the Consensus -1 Ribosomal Frameshifting Signals Have Been Linked to Disease.

Description	Diseases and allelic variants*.
ETFA-electron transfer flavoprotein α-subunit precursor	Type II glutaricaciduria. Note: allelic variant .0004 (Val270DEL3bp) disrupts the spacing between the slippery site and the RNA pseudoknot.
Triacylglycerol lipase	Lipoprotein Lipase Deficiency. Note: allelic variant .0027(Arg75Ser) disrupts stem 1 of the RNA pseudoknot. Familial Chylomicronemia Syndrome. Note: allelic variant .0021 (Trp86Arg) disrupts stem 2 of the RNA pseudoknot.
FASL receptor	Autoimmune lymphoproliferative syndrome. Note: allelic variant .0007 (Tyr216Cys) disrupts stem2 in the RNA pseudoknot.

**The human diseases that are known to be linked to these genes. References to these can be found in the Online Mendelian Inheritance in Man (OMIM) database on the WorldWideWeb.*

In the human gene encoding triacylglycerol lipase, the .0027 allelic variant of triacylglycerol lipase (linked to lipoprotein lipase deficiency) (Wilson et al., 1993), and the .0021 allelic variant (linked to Familial Chylomicronemia Syndrome) (Gotoda et al., 1992) are both predicted to disrupt the RNA pseudoknot component of the consensus -1 ribosomal frameshift signal. Similarly, the .0007 allelic variant of the FASL antigen (linked to autoimmune lymphoproliferative syndrome) (Bettinardi et al., 1997) is also predicted to disrupt the RNA pseudoknot. Disruption of the mRNA pseudoknot is predicted

to abolish programmed -1 ribosomal frameshifting (reviewed in Dinman, 1995; Jacks, 1996; Farabaugh, 1996; Brierley, 1995; Gesteland and Atkins, 1996; Dinman et al., 1998; TenDam et al., 1990). In addition, the .0004 allele of the ETFA-electron transfer flavoprotein α-subunit precursor (linked to type II glutaricaciduria) (Freneaux et al., 1992) disrupts the spacing between the slippery site and the RNA pseudoknot, which is predicted to result in a decrease in programmed -1 ribosomal frameshifting efficiency (Dinman and Wickner, 1992; Brierley et al., 1991; Brierley et al., 1992; Morikawa and Bishop, 1992).

In summary, a computer implemented method has been developed that is capable of detecting known viral -1 ribosomal frameshift signals. We have demonstrated that these motif hits occur with frequencies approximately one order of magnitude greater than random in many large DNA sequence databases, and there are examples where the consensus frameshift signals appear to be evolutionarily conserved in homologous genes in different organisms. Finally, three examples are shown where single missense mutations that occur in the frameshift signal correspond with previously identified genetically inherited diseases in humans.

Computer identified motif hits can promote efficient levels of programmed -1 ribosomal frameshifting in *S. cerevisiae*.

Using a series of frameshift reporter plasmids and yeast strains previously developed, a set of motif hits that were identified by the computer program were tested for ability to promote efficient levels of programmed -1 ribosomal frameshifting in intact cells. Plasmids to monitor programmed ribosomal frameshifting were previously described (Cui et al., 1996; Dinman et al., 1997;m Dinman and Kinzy, 1997; Tumer et al., 1998; Cui et al., 1998). Briefly, in all of these plasmids, transcription is driven from the yeast PGK1 promoter into an AUG translational start site. The *E. coli lacZ* gene serves as the reporter, and transcription termination utilizes the yeast PGK1 transcriptional terminator. In the p0 plasmids, lacZ is in the 0-frame with respect to the translational start site, and measurement of β-galactosidase activity generated from cells transformed with these plasmids serve as the 0-frame controls. In the p-1 series, the predicted programmed -1 ribosomal frameshift signals have been cloned into unique Bam HI and Sma I sites in p0. Thus, in the p-1 series of plasmids, lacZ is in the -1 frame with respect to the translational start site, and is 3' of a predicted programmed -1 ribosomal frameshift signal such that β-galactosidase can only be produced as a consequence of a programmed -1 ribosomal frameshift. p0 and p-1 are introduced into yeast cells in parallel, and the amount of the lacZ gene product (β-galactosidase) present in both sets of cells are determined. Motif hits amplified by PCR from yeast genomic DNA were cloned into pJD160 in such a way that a programmed -1 ribosomal frameshift is required for translation of the *lacZ* gene. This set constitutes the frameshift test plasmids. Programmed -1 ribosomal frameshift efficiencies were calculated by dividing the β-galactosidase activities generated

from cells harboring frameshift test plasmids by the β-galactosidase activity generated by the 0-frame control, pJD160. As a control to determine the background levels of unprogrammed -1 frameshifting, β-galactosidase activities generated from cells harboring pJD160.-1 were determined. Further, the efficiency of programmed -1 ribosomal frameshifting as promoted by the L-A virus frameshift signal was determined in order to compare the frameshift promoting abilities of the motif hits to a known programmed -1 ribosomal frameshift signal. The results of these experiments demonstrate that the motif hits that were tested are all capable of promoting efficient programmed -1 ribosomal frameshifting as compared to the L-A frameshift signal (Table 4).

Table 4. Motif hits can promote efficient levels of programmed -1 ribosomal frameshifting in intact yeast cells.

Frameshift signal	% -1 ribosomal frameshifting
L-A dsRNA virus	1.9%
RAS1	4.4%

Discussion

Following the hypothesis that biological systems tend to conserve usable regulatory mechanisms, a computer program was developed based on an algorithm describing a set of consensus programmed -1 ribosomal frameshift signals. It has been demonstrated 1) that the program is capable of finding known frameshift signals, 2) that these motif hits occur in the large DNA databases at frequencies that are significantly greater than random, 3) that very similar motif hits can be found to be evolutionarily conserved in homologous genes from different species, 4) that known missense alleles that have been linked to human diseases are predicted to disrupt frameshift signals, and 5) that at least one motif hit from the yeast *S. Cerevisiae* genome is capable of promoting efficient levels of programmed -1 ribosomal frameshifting. These findings indicate that, in addition to viruses, programmed -1 ribosomal frameshifting is also utilized to regulate the expression of chromosomally encoded genes in eukaryotes.

Possible regulatory roles of programmed -1 ribosomal frameshifting.

There are three possible translational outcomes of a programmed ribosomal frameshift. A frameshift could result in the production of an extended fusion protein such as the viral gag-pol protein. In the context of cellular proteins, there are many imaginable consequences of the addition of a C-terminal domain. For example, such a domain could provide a means to physically localize the protein to a different compartment. An additional C-terminal domain could encode an enzymatic or signaling function, or even provide an autoregulatory function. A programmed ribosomal frameshift could also

result in the production of two proteins having identical N-terminal domains and different C-termini. In addition to the consequences listed above, such an outcome could also result in a bifurcation function. For example, the two proteins could have identical input functions (e.g. can both act as a receptor for the same ligand), but different output functions (e.g. transduction of the signal to different regulatory pathways). Thus, programmed ribosomal frameshifting could be utilized by cells to effect activity in different biological regulatory pathways.

A third possible outcome is that programmed ribosomal frameshifting results in a premature termination event. Such an event may signal to the translational complex that the mRNA being translated contains a nonsense mutation. mRNAs which contain nonsense mutations are rapidly degraded via the nonsense-mediated mRNA decay (NMD) pathway (reviewed in Weng et al., 1997). The rate of mRNA decay plays an important role in the regulation of gene expression, and the decay rate of an mRNA can be modulated, depending on the cell type, stage of the cell cycle, or environmental conditions (see Atwater et al., 1990; Cleveland and Yen, 1989; Peltz et al., 1991 for reviews). It has been shown that aberrant regulation of post-transcriptional control mechanisms can lead to disease (reviewed in Ross, 1995). Altered stability of certain mRNAs has been suggested to be an important factor in determining the onset and severity of disease. Examples include the differences in the stability between the wild-type c-*myc* mRNA and its tranlocated formed found in Burketts lymphoma; between the highly oncogenic v-*fos* mRNA and its weakly oncogenic c-*fos* mRNA (reviewed in Weng et al., 1997; Lee et al., 1988; Raymond et al., 1989) and between mRNAs encoding the oncogenic E6/E7 proteins of the nonintegraed human papilomaviruses found in benign cervical lesions and the more stable E6/E7 mRNAs synthesized from the integrated form of the virus that correlates with cervical carcinomas (Jeon and Lambert, 1995). Further, mutations in *trans*-acting factors that regulate mRNA turnover may also lead to aberrant gene regulation and disease. Mutations in *trans*-acting factors specifically stabilize the lymphokine GM-CSF mRNA in monocytic tumors compared with non-tumor cells (Schuler and Cole, 1988).

As noted above, both the *RAS1* and STE5 programmed ribosomal frameshift signals fall into this class, promoting approximately 5% of translating ribosomes to encounter premature termination signals. One concern is the biological significance of a mere 5% efficiency of frameshifting in that is this would result in an insignificant 5% change in overall Ras1 protein concentrations. However, this does not take into account the fact that a -1 ribosomal frameshift would lead to the premature translational termination of that specific mRNA molecule. As such, a frameshift event on a specific mRNA would trigger the destruction of that mRNA, and thus these frameshift signals should act as mRNA destabilizing elements, decreasing the overall stability of all of those mRNAs. For example, in the absence of a frameshift signal, each mRNA might be translated 100 times, resulting in the production of 100 protein molecules per mRNA. In the presence of the signal however, a frameshift efficiency of 5% would result in 1 in 20

translating ribosomes encountering a premature termination signal on each individual mRNA, activating NMD pathway. Thus, each mRNA would be limited to producing an average of only 19 of 20 protein molecules, an 80% reduction in the total amount of protein synthesized. Thus we propose that programmed ribosomal frameshifting may be used by a subset of cellular mRNAs as a general mechanism to regulate their stability and consequently the abundance of their encoded protein products.

The abundance of a subset of cellular mRNAs may be biologically regulated by modulation of programmed -1 ribosomal frameshifting efficiencies. As noted above, the rate of mRNA decay plays an important role in the regulation of gene expression, and the decay rate of an mRNA can be modulated, depending on the cell type, stage of the cell cycle, or environmental conditions. Thus, programmed -1 ribosomal frameshifting may be used as a mechanism to regulate the abundance of a subset of cellular mRNAs. The possibilities for signaling mechanisms that may act to modulate programmed -1 ribosomal frameshift efficiencies are numerous. These may include the cell-cycle, heat shock, and developmental, and other signals.

The recent observation that anisomycin specifically inhibits programmed -1 ribosomal frameshifting (Dinman et al., 1997) provides a potentially intriguing link between regulation of programmed ribosomal frameshifting and the control of cell growth and division. There is a considerable body of literature describing the ability of anisomycin to activate the Jun kinase/stress-activated protein kinase (JNK/SAPK) pathway (reviewed in Shu et al., 1996; Moxham et al., 1996). Anisomycin stimulates expression of the c-*jun*, c-*fos* and c-*myc* proto-oncogenes (Yu et al., 1996; Moxham et al., 1996; Kawasaki et al., 1996; Hazzalin et al., 1996), activates the MAP-kinases (Moxham et al., 1996; Hazzalin et al., 1996; Nahas et al., 1996; Cano et al., 1996), pre-ribosomal S6, histone H3 and HMG-14 (Hazzalin et al., 1996), ELAM-1 (Gersa et al., 1992), angiotensin II (Yu et al., 1996), the Ras-dependent and Ras-independent pathways (Kawasaki et al., 1996), p38/RK (yeast Hog1p) (Nahas et al., 1996; Cano et al., 1996), MEK6 (Stein et al., 1996), and insulin-like growth factor II (Nielsen et al., 1995). The effects of anisomycin are specific: other protein synthesis inhibitors (*e.g.* cycloheximide or emetine) block cell cycle progresssion without strong JNK/SAPK induction (Shu et al., 1996).

Anisomycin inhibits protein translation at the level of elongation. It has been proposed that inhibition of protein synthesis leads to a decrease in the levels of labile negative growth regulating proteins, thus promoting cell growth and division (Gersa et al., 1992; Smailov et al., 1993; Rosenwald et al., 1995; Abdelmajid et al., 1993). According to this hypothesis however, any general inhibitor of translation should result in this effect, and thus the JNK/SAPK pathway should be nonspecifically induced by any inhibitor of protein synthesis. This is not the case since 1) not all translational inhibitors stimulate this pathway, and 2) pathway-specific induction is observed. Since anisomycin decreases the efficiency of programmed -1 ribosomal frameshifting efficiencies, it is believed that the regulation of expression of proteins involved in the JNK/SAPK signaling pathway occurs at the post-transcriptional

level by regulating efficiencies of ribosomal frameshifting rather than by generally inhibiting protein synthesis. This model retains the suggestion that there is a labile element tied to specific inhibitors of protein synthesis, but that it is mRNA instead of protein. Thus, anisomycin likely causes an increase in the abundance of these labile cellular mRNAs which encode positive growth regulators by decreasing programmed ribosomal frameshifting efficiencies. In normal growth these mRNAs would promote ribosomes to shift reading frame into early termination codons, making these mRNAs substrates for the nonsense-mediated mRNA decay pathway. These mRNAs would normally be non-abundant species with short half-lives and low production of their encoded protein products. However, under certain conditions, they could be stabilized as a consequence of decreased efficiencies of ribosomal frameshifting. Stabilization of these mRNAs would upregulate the expression of their encoded products, which presumably are positive regulators of cell growth and division. The ability to specifically regulate the half-lives, and thereby the abundance, of mRNAs containing -1 ribosomal frameshift signals provides the cell with a level of specificity that the labile negative growth regulating protein model cannot account for.

Several lines of evidence are consistent with this model. First, anisomycin should stabilize nonsense-mRNAs. It has been demonstrated that anisomycin acts post-transcriptionally by stabilizing the ELAM-1 mRNA and other nonsense-containing mRNAs (Gersa et al., 1992; Li et al., 1996), and that anisomycin regulates the expression of prepro-IGF-II in a post-transcriptional manner (Nielsen et al., 1995). Second, if anisomycin induces cell proliferation by decreasing -1 ribosomal frameshifting efficiencies in a specific set of mRNAs, then sparsomycin should have anti-proliferative effects by virtue of its ability to increase -1 ribosomal frameshifting efficiencies (see Dinman et al., 1997). Sparsomycin analogs have been demonstrated to have antitumor activities (Hofs et al., 1995a; Hofs et al., 1995b; Hofs et al., 1994) Third, in three of the well characterized examples of non-viral programmed ribosomal frameshifting, all involve autoregulatory feedback mechanisms where levels of the encoded protein products affect the efficiencies of ribosomal frameshifting along their own mRNAs (reviewed in Gesteland and Atkins, 1996). These examples where ribosomal frameshifting efficiency is autoregulated provide further support for the hypothesis that programmed ribosomal frameshifting can be used to regulate the abundance and expression of cellular mRNAs and their encoded products.

All of the publications cited herein or listed below are cited for background purposes and the disclosure of such publications is not essential for an understanding of the invention. All of the publications are hereby incorporated by reference.

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What is claimed is:

1. A method of identifying a nucleic acid sequence involved in ribosomal frameshifting comprising:

1) searching a database of gene sequences to identify sequences which contain the sequence XXX YYY Z, wherein

XXX represents GGG, AAA, TTT or CCC,

YYY represents AAA or TTT,

Z represents A, T, or C

and wherein XXXYYYZ is not AAAAAAA or TTTTTTT;

2) further searching among those sequences identified in step 1 for a sequence encoding a pseudoknot structure which is within eight nucleotides of the sequence identified in step 1.

2. The method of claim 1, wherein XXXYYYZ represents a sequence selected from the group consisting of GGG AAA A, GGG AAA T, GGG AAA C, AAA AAA T, AAA AAA C, TTT AAA A, TTT AAA T, TTT AAA C, CCC AAA A, CCC AAA T, CCC AAA C, GGG TTT A, GGG TTT T, GGG TTT C, AAA TTT A, AAA TTT T, AAA TTT C, TTT TTT A, TTT TTT C, CCC TTT A, CCC TTT T, and CCC TTT C.

3. A method of identifying a nucleic acid sequence involved in ribosomal frameshifting comprising the steps of:

selecting a gene sequence having a sequence of nucleotides from the group of GGG, AAA, TTT and CCC;

selecting said gene sequence having an adjacent sequence of nucleotides from the group of AAA and TTT;

selecting said gene sequence having a nucleotide from the group of A, T and C, said nucleotide adjacent to said adjacent sequence of nucleotides;

excluding said gene sequence wherein said sequence of nucleotides is AAA, said adjacent sequence of nucleotides is AAA and said nucleotide is A;

excluding said gene sequence wherein said sequence of nucleotides is TTT, said adjacent sequence of nucleotides is TTT and said nucleotide is T;

searching for an encoded pseudoknot structure which starts within eight nucleotides of said selected gene sequence.

4. The method of claim 3 wherein XXXYYYZ represents a sequence selected from the group consisting of GGG AAA A, GGG AAA T, GGG AAA C, AAA AAA T, AAA AAA C, TTT AAA A,

TTT AAA T, TTT AAA C, CCC AAA A, CCC AAA T, CCC AAA C, GGG TTT A, GGG TTT T, GGG TTT C, AAA TTT A, AAA TTT T, AAA TTT C, TTT TTT A, TTT TTT C, CCC TTT A, CCC TTT T, and CCC TTT C.

5. A system for identifying a nucleic acid sequence involved in ribosomal frameshifting, the system comprising:

access means for accessing a database of gene sequences;

selection means for selecting a particular gene sequence from said database of gene sequences, said particular gene sequence having a sequence of nucleotides from the group of GGG, AAA, TTT and CCC, an adjacent sequence of nucleotides from the group of AAA and TTT, a nucleotide from the group of A, T and C, said nucleotide adjacent to said adjacent sequence of nucleotides, wherein said particular gene sequence is excluded from selection when said sequence of nucleotides is AAA, said adjacent sequence of nucleotides is AAA and said nucleotide is A and said particular gene sequence is excluded from selection when said sequence of nucleotides is TTT, said adjacent sequence of nucleotides is TTT and said nucleotide is T;

pseudoknot search means for locating an encoded pseudoknot structure which starts within eight nucleotides of said selected gene sequence.

6. The system as recited in claim 5 wherein XXXYYYZ represents a sequence selected from the group consisting of GGG AAA A, GGG AAA T, GGG AAA C, AAA AAA T, AAA AAA C, TTT AAA A, TTT AAA T, TTT AAA C, CCC AAA A, CCC AAA T, CCC AAAC, GGG TTT A, GGG TTT T, GGG TTT C, AAA TTT A, AAA TTT T, AAA TTT C, TTT TTT A, TTT TTT C, CCC TTT A, CCC TTT T, and CCC TTT C.

7. A method of regulating expression of a mammalian gene comprising modulating the frequency of ribosomal frameshifting during translation of messenger RNA.

8. The method according to claim 7, wherein the frequency of frameshifting is increased.

9. The method according to claim 7, wherein the frequency of frameshifting is decreased.

10. The method according to claim 7, wherein the gene encodes an oncogene.

11. The method according to claim 7, wherein the gene encodes a tumor suppresser gene.

12. The method according to claim 7, wherein the gene encodes a hormone.

13. The method according to claim 7, wherein the gene encodes a human growth hormone.

14. The method according to claim 7, wherein the gene encodes a hormone receptor.

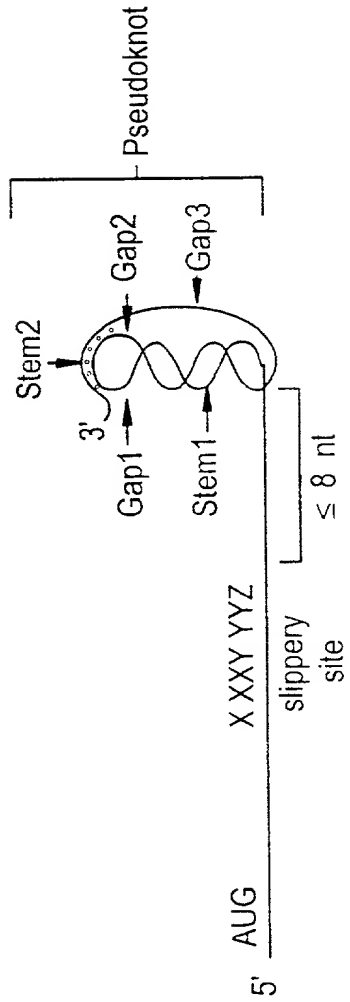
15. The method according to claim 7, wherein the gene encodes a human growth hormone receptor.

16. The method according to claim 6, wherein the gene encodes a catalytic enzyme.

17. A method of treating a disease caused by reduced expression of a gene product which is produced as a result of ribosomal frameshifting, comprising increasing the frequency of ribosomal frameshifting during translation of the gene.

18. A method of treating a disease caused by increased expression of a gene product which is produced as a result of ribosomal frameshifting, comprising decreasing the frequency of ribosomal frameshifting during translation of the gene.

FIG. 1


Pseudoknot
Stem2
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Gap3
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Gap1
Stem1
≤ 8 nt
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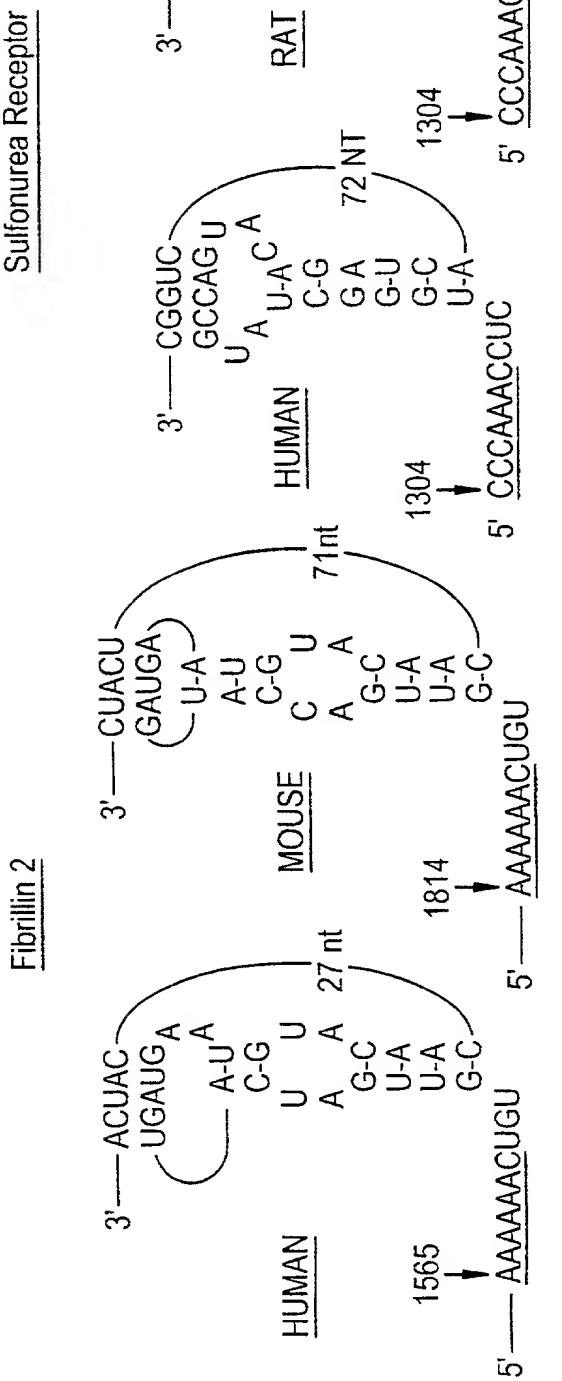
FIG. 2


Sulfonurea Receptor
Fibrillin 2
HUMAN
MOUSE
RAT
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1814
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71nt
72 NT
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CUACU
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CCCAAACCUC

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: **Dinman et al.**		
International Application No. **PCT/US99/20942**	Group Art Unit:	**Not Assigned**
International Filing Date **September 13, 1999**	Examiner:	**Not Assigned**
U.S. Filing Date: **March 13, 2001**		
For: **RIBOSOMAL FRAMESHIFT TARGETS**		

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

POWER OF ATTORNEY TO ASSOCIATE ATTORNEY

Please recognize Kristine L. Butler (Reg. No. 42,376) as Associate Attorney in this application.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Declaration and Power of Attorney

As the below named inventors, we hereby declare that:

Our residence, post office address and citizenship are as stated below next to our name.

We hereby claim the benefit under Title 35, United States Code, 119(e) of any United States provisional application(s) identified below:

U.S. Serial Number 60/100,285 filed September 14, 1998.

We believe we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled **RIBOSOMAL FRAMESHIFT TARGETS** filed on September 13, 1999 as U.S. Serial No. 09/787,072.

We hereby state that we have reviewed and understand the contents of the above identified specification, including the claims, as amended by an amendment, if any, specifically referred to in this oath or declaration.

We acknowledge the duty to disclose all information known to us which is material to patentability as defined in Title 37, Code of Federal Regulations, 1.56.

We hereby claim foreign priority benefits under Title 35, United States Code, 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

None.

We hereby claim the benefit under Title 35, United States Code, 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, 112, we acknowledge the duty to disclose all information known to us to be material to patentability as defined in Title 37, Code of Federal Regulations, 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

PCT Application No. PCT/US99/20942 filed September 13, 1999.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

We hereby appoint as our attorneys or agents the registered persons identified under

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for the law firm of Gibbons, Del Deo, Dolan, Griffinger & Vecchione, said attorneys or agents with full power of substitution and revocation to prosecute this application and transact all business in the Patent and Trademark Office connected herewith.

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